

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT 2004
(PCT Article 36 and Rule 70)

WIPO PCT

Applicant's or agent's file reference A-157453	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/PEA/416)	
International application No. PCT/ES 03/00379	International filing date (day/month/year) 23.07.2003	Priority date (day/month/year) 25.07.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/68		
Applicant FUNDACIO PRIVADA I INSTITUT DE RECERCA DE ...et al		

BEST AVAILABLE COPY

- This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
- This REPORT consists of a total of 6 sheets, including this cover sheet.



☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of ~~11~~ sheets.

12

- This report contains indications relating to the following items:

- I ☒ Basis of the opinion
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☒ Lack of unity of invention
- V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 18.02.2004	Date of completion of this report 29.10.2004
Name and mailing address of the International preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Hennard, C Telephone No. +49 89 2399-7355 

INTERNATIONAL PRELIMINARY
EXAMINATION REPORT

International application No. PCT/ES 03/00379

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17):*

Description, Pages

5-15 as originally filed
1, 1a, 2, 2a, 3, 3a, 4, 4a received on 19.08.2004 with letter of 18.08.2004

Claims, Numbers

1-9 received on 19.08.2004 with letter of 18.08.2004

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
☐ the language of publication of the international application (under Rule 48.3(b)).
☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
☐ filed together with the international application in computer readable form.
☒ furnished subsequently to this Authority in written form.
☒ furnished subsequently to this Authority in computer readable form.
☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/ES 03/00379

**BEST
AVAILABLE
COPY**

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees, the applicant has:

- ☐ restricted the claims.
- ☐ paid additional fees.
- ☐ paid additional fees under protest.
- ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

- ☐ complied with.
- ☒ not complied with for the following reasons:

see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

- ☒ all parts.
- ☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	1-9
	No: Claims	None
Inventive step (IS)	Yes: Claims	None
	No: Claims	1-9
Industrial applicability (IA)	Yes: Claims	1-9
	No: Claims	None

2. Citations and explanations

see separate sheet

Re Item

Lack of unity of invention

1. **Unity of invention (Rule 13 PCT):**

The arguments put forwards by the applicant have been taken into consideration and the IPEA came to the following conclusion:

From the arguments it is understood that the common linking concept between the different allelic variants is in the fact that they affect the functionality of the factor VII but also the levels at which the protein is found.

Nevertheless, the prior art also refers to the mutations in the factor VII gene and correlates this mutation with a reduced plasma level of the protein (see especially **D3**). Therefore, the argument are not considered as convincing and the objection for lack of unity is maintained and is as follows:

Claim 1 of the present application concerns nucleic acid variants of the gene coding for factor VII, the variants being characterised by the mutations as listed in table 1. **D1-D5** disclose factor VII gene variants responsible for the production of the deficient protein essential for the initiation phase of normal haemostasis. Because the concept of factor VII gene variants inducing the production of deficient proteins leading to cardiovascular diseases is known, no common inventive concept for the different variants claimed can be found. Therefore, the present **claim 1** concerns different solutions to the single problem of finding new variants producing the deficient protein, all these solutions being not so linked as to form a single inventive concept (**Rule 13 PCT**). It is concluded that each variant listed in table 1 represents a different solution and thereby a distinct invention.

Further, it is brought to the applicant's attention that according to the new PCT guidelines (see **Guidelines Part III, 10.54**) there is no unity of invention between alternative chemical compounds (in the present case nucleic acid molecules having polymorphisms at given positions) if they do not share a significant structural element that is essential to the common property or activity.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

- D1: THROMBOSIS RESEARCH, vol. 98, 2000, pages 9-17
D2: HUMAN MUTATION, vol. 15, n° 6, 2000, pages 489-496
D3: BLOOD, vol. 93, n° 10, 1999, pages 3432-3441
D4: ARTERIOSCLEROSIS AND THROMBOSIS, vol. 11, n° 3, 1991, pages 540-546
D5: HUMAN GENETICS, vol. 90, n° 5, 1993, pages 575-576

2. **Novelty (Article 33(2) PCT):**

By deleting the allelic variant having a T/C substitution at position -122 from the list of variants of **claim 1**, novelty has been established. Therefore, since the allelic variants listed in the claims are not disclosed in the **D1-D5**, **claims 1-9** are considered novel and fulfil the requirements of **Article 33(2) PCT**.

3. **Inventive merit (Article 33(3) PCT):**

3.1 The argumentation as well as the "enclosure 2" provided by the applicant to justify an inventive merit have been taken into consideration. The following is nevertheless to be considered.

In the "enclosure 2" on page 13, last paragraph it is stated that "*Obviously, it is very important to unravel the nature of these polymorphisms (allelic variants of the application) with biological studies of in vitro expression to exactly characterise the nature of these variants, to confirm their functionality, and to distinguish among the markers in the three highly correlated clusters*".

According to this statement it appears that it is not clear which allelic variants (if any) really are responsible for the disease and are susceptible to be used in the manufacture of a medicament. Therefore, the objection for lack of inventive merit of the claims is maintained as follows:

3.2 **D2**, which is considered to be the closest prior art, concerns various mutations of the factor VII gene leading to deficiency of the protein, essential for the initiation phase of normal haemostasis. The oligonucleotides molecules of present **claim 1** distinguish themselves from the mutations of **D2** by position in the gene for the mutation. No technical effect is achieved by these different allelic variants, therefore, the problem to be solved by the present **claim 1** is to provide new allelic variants affecting the activity of factor VII.

No indication in the prior art documents **D1-D5** taken separately or together, is to be found that would allow the skilled person to anticipate which region of the gene and which polymorphism could induce the alteration of the factor VII protein.

Nevertheless, in the absence of evidence that the variants listed in the present table 1 do solve the problem of providing new polymorphisms demonstrating

BEST AVAILABLE COPY

1 that the activity of factor VII is altered by the polymorphism (see item 5.1 below), it is considered that the variants do not solve the problem of the application. Therefore, **claim 1** of the present application is considered not to fulfil the requirements of **Article 33(3) PCT**.

3.3 Following the same reasoning for **claims 3-7** leads to the conclusion that these claims do not introduce any feature susceptible of an inventive merit over **D2** since this document does also disclose probes used for the detection of mutations in the factor VII gene.

3.4 Furthermore, since it is not clear which variants of table 1 will lead to a defective factor VII protein, it is not possible to determine, at this stage, which of these variants are suitable for the development of a therapeutic, preventive or diagnostic approach or for the manufacture of a medicament. Therefore, **claims 2 and 8-9** are at present not considered to fulfil the requirements of **Article 33(3) PCT**.

3.5 It is concluded that **claims 1-9** of the present application do not fulfil the requirements of **Article 33(3) PCT**.

4. **Industrial applicability (Article 33(4) PCT):**

Due to the nature of the claims, an industrial applicability of the invention is obvious and **claims 1-9** are considered to fulfil the requirements of **Article 33(4) PCT**.

5. In the absence of results showing that the variants of the present application are responsible for the production of a deficient protein of factor VII (mutation of a gene is a statistical event which does not necessarily lead to the disfunction of the corresponding protein), it is considered that **claim 9** lacks support (**Article 6 PCT**) since it is not clear which variant could have a therapeutical effect.

6. **Claims 8 and 9** relate to the use of nucleic acid molecules for the preparation of medicament or approaches for the treatment of cardiovascular diseases. Since cardiovascular diseases encompass a group of diseases, the scope of these claims is unclear (**Article 6 PCT**). - error tipográfico - quitar -s.

NEW ALLELIC VARIANTS IN THE FACTOR VII GENE

FIELD OF THE INVENTION

5 The present invention relates to the field of cardiovascular diseases.

 In particular, the present invention relates to
the identification of new allelic variants in the factor
VII gene sequence for determining the predisposition to a
10 cardiovascular disease.

BACKGROUND OF THE INVENTION

Factor VII is a vitamin K-dependent glycoprotein
15 synthesized in the liver and secreted into the blood as an
inactive zymogen at a concentration of 0.5 µg/ml⁴ (~~Fair~~
~~Blood, 1983~~⁴). Following endothelial damage, the tissue
factor (TF) is exposed and it binds to the Factor VII,
setting up a coagulation reaction (~~Peterud, Proc. Natl.~~
20 ~~Acad. Sci. USA, 1977; Bauer et al., Blood, 1990~~^{2,3}).

The gene that encodes the Factor VII is located on
13q34-q.ter (~~Pfeiffer et al., 1982; Gilgenkrantz et al.,~~
~~1980~~^{4,5}), contains 9 exons and 8 introns of 12.8 kb and codes
for a protein of 406 amino acids. The complete gene
25 sequence for human Factor VII was determined by O'Hara et
al. (O'Hara P.J. et al., "Nucleotide sequence of the gene
coding for human factor VII, a vitamin K-dependent protein
participating in blood coagulation"; Proc. Natl. Acad.
Sci. U.S.A. 84:5158-5162 (1987)). The mRNA is
30 polyadenylated in multiple positions and has an efficient
differential splicing. The mature protein has a molecular
mass of approximately 50 KDa.

The activated form of the factor VII consists on
one heavy chain and one light chain, both coded by the
35 same gene, and linked by a disulphur bond between the

[1]= Fair D.S. Quantitation of factor VII in the plasma of normal and warfarin-treated individuals by radioimmunoassay. Blood 1983; 62: 784-91.

[2]= Osterud B., Rapaport S.I Activation of factor IX by the reaction product of tissue factor and factor VII: additional pathway for initiating blood coagulation. Proc. Natl. Acad. Sci. USA 1977; 74: 5260-4.

[3]= Bauer K.A., Kass B.L., ten Cate H., Hawiger J.J, Rosenberg R.D. Factor IX is activated in vivo by the tissue factor mechanism. Blood 1990; 76: 731-736.

[4]= Pfeiffer, R. A.; Ott, R.; Gilgenkrantz, S.; Alexandre, P. Deficiency of coagulation factors VII and X associated with deletion of a chromosome 13 (q34): evidence from two cases with 46,XY,t(13;Y)(q11;q34). Hum. Genet. 1982; 62: 358-360

[5]= Gilgenkrantz, S.; Briquel, M.-E.; Andre, E.; Alexandre, P.; Jalbert, P.; Le Marec, B. et al. Structural genes of coagulation factors VII and X located on 13q34. Ann. Genet. 1986; 29: 32-35

cysteine 135 and the cysteine 262 (~~Hagen et al., 1985~~)^{<6>}. It contains two EGF domains (epidermal growth factor domain), one Gla domain (γ -carboxyglutamic acid domain) and one trypsin-like catalytic domain (~~Hagen et al., Natl. Acad. Sci. USA, 1984~~)^{<7>}.

The heavy chain includes the catalytic part of the molecule and the heavy chain contains the Gla domain involved in the Ca^{2+} binding and the membrane binding, which are essential for factor VII activity.

10 The factor VII heavy-chain variants involve the direct interference in the activation process or the interruption of the catalytic mechanism, whereas most of the light-chain variants interrupt the interactions with Ca^{2+} or with membrane components which results in
15 dysfunctional molecules (~~Zheng et al., Blood Coagul. Fibrinol, 1994~~)^{<8>}.

Hereditary factor VII deficiency is an uncommon disorder showing autosomal recessive inheritance with high penetrance and variable expressivity (~~Kapfer et al., 1960~~)^{<9>}.
20 (~~Triplet et al., 1985~~)^{<10>}. It has an incidence of 1 per 500,000 in the general population (~~Wulff and Hermann, Hum. Mutation 15, 2000~~)^{<11>} and was recognized for the first time by Alexander et al., 1951. Some of the factor VII gene mutations have been identified and affect all domains of
25 the protein, although about 50% of said mutations affect the protease domain (~~Wulff and Hermann, Hum. mutation, 2000~~)^{<12>}, which indicates that the loss of protease function is the main cause of factor VII deficiency.

In general, the most common forms of disorder
30 involve the presence of dysfunctional factor VII, which consists in low antigen levels in the plasma and a lengthening of prothrombin time due to defective activity of these molecules.

An absence of factor VII activity in plasma causes
35 severe haemorrhage shortly after birth; indeed, there are

[6], [7]= Hagen, F. S.; Gray, C. L.; O'Hara, P.; Grant, F. J.; Saari, G. C.; Woodbury, R. G.; Hart, C. E.; Insley, M.; Kisiel, W.; Kurachi, K.; Davie, E. W. : Characterization of a cDNA coding for human factor VII. Proc. Nat. Acad. Sci. 83: 2412-2416, 1986

[8]= Zheng DQ, Shurafa M, James HL. Factor VII G331D: a variant molecule involving replacement of a residue in the substrate-binding region of the catalytic domain. Blood Coagul Fibrinolysis. 1996 Jan;7(1):93-6.

[9]= Kupfer, H. G.; Hanna, B. L.; Kinne, D. R. : Congenital factor VII deficiency with normal Stuart activity: clinical, genetic and experimental observations. Blood 15: 146-163, 1960.

[10]= Triplett, D. A.; Brandt, J. T.; Batard, M. A. M.; Dixon, J. L. S.; Fair, D. S. : Hereditary factor VII deficiency: heterogeneity defined by combined functional and immunochemical analysis. Blood 66: 1284-1287, 1985.

[11], [12]= Wulff, K.; Herrmann, F. H. : Twenty two novel mutations of the factor VII gene in factor VII deficiency. Hum. Mutat. 15: 489-496, 2000.

studies in which mice deficient in FVII due to targeted disruption of the factor VII gene suffered fatal haemorrhage in the peri-partum period (~~MeVey et al., Hum. Mutation, 2004~~)^{<13>}.

5 Moreover, about 30-40% of the variation of FVIIa levels in the general population can be explained by the existence of polymorphisms in the FVII gene (~~Bernardi et al., Blood 1996~~)^{<14>}. These polymorphisms or allelic variants nevertheless show different allelic frequencies in
10 different populations (~~Green et al., Arterioscler. Thromb., 1991; Bernardi, Marchetti, Pinotti, Arterioscler. Thromb. Vasc. Biol., 1996~~)^{<15,16>}.

These allelic variants have been associated with varied risk of suffering from cardiovascular diseases, although the studies in which such an association has been
15 described are contradictory and in no instance conclusive (~~Sirelli et al., New England J. Med., 2000; Iacoviello et al., N. Eng. J. Med., 1998~~)^{<14,18>}. Furthermore, all the studies suffer from design errors and lack of statistical power.

20 The design and methodology used to date to approach the study of cardiovascular disease were based on investigating for presence of the risk factor in healthy individuals (controls) and disease sufferers (cases) who were unrelated to each other. Where the hypothetical risk
25 factor was observed more frequently (in statistical terms) in the cases than in the controls it was concluded that the disease was associated with the factor under study. Strictly, however, a relationship of association does not necessary imply causation. This type of study, so-called
30 Association or Case/Control Study, is entirely unsuitable for investigating genetic causes of complex illnesses, such as cardiovascular disease (~~Gambaro et al., Lancet 2000~~)^{<19>}. Conventional epidemiological studies basically serve to identify environmental causes of illness (such as
35 the smoking habit and lung cancer, oral contraceptives and

[13]= McVey J.H, Boswell E, Mumford A.D, Kemball-Cook G, Tuddenham E.G.D. Factor VII Deficiency and the FVII Mutation Database. Hum. Mutat. 2001; 17: 3-17.

[14]= Bernardi F., Marchetti G., Pinnotti M., Arcieri P., Baroncini C., Papacchini M., Zeponi E., Ursicino N., Chiarotti F.M. (1996) Factor VII gene polymorphisms contribute about one third of the factor VII level variation in plasma. Arterioscler. Thromb. Vasc. Biol., 16, 72-76.

[15]= Green F., Kelleher C., Wilkes H., Temple A., Meade T., Humphries S. (1991) A common genetic polymorphism associated with lower coagulation factor VII levels in healthy individuals. Arterioscler. Thromb., 11, 540-546.

[16]= Bernardi F, Castaman G, Pinotti M, Ferraresi P, Di Iasio MG, Lunghi B, Rodeghiero F, Marchetti G. Mutation pattern in clinically asymptomatic coagulation factor VII deficiency. Hum Mutat. 1996;8(2):108-15.

[17]= Girelli D, Russo C, Ferraresi P, Olivieri O, Pinotti M, Friso S, et al. Polymorphisms in the factor VII gene and the risk of myocardial infarction in patients with coronary artery disease. N.Engl.J.Med. 2000; 14: 774-80.

[18]= Iacoviello L.; DiCastelnuovo A.; DeKnijff P.; D' Orazio A., Amore C., Arboretti R. et al Polymorphisms in the coagulation factor VII gene and the risk of Myocardial infarction. N. Eng. J. Med. 1998; 338: 79-85.

[19]= Gambaro G, Anglani F, D'Angelo A. Association studies of genetic polymorphisms and complex disease. Lancet. 2000 Jan 22;355(9200):308-11.

venous thrombosis, or a vitamin-C-deficient diet and scurvy), but are highly ineffective when it comes to locating the genes involved. However, owing to the widespread use of PCR techniques in clinical laboratories, there are large numbers of Association Studies that relate genetic variants (polymorphisms) in certain candidate genes with all kinds of illnesses. Much confusion has been caused, because the results relating to a single polymorphism are often contradictory. Neither has the study of cardiovascular disease, for both venous and arterial types, remained free from this methodological perversion nor from the attendant chaotic collection of results (~~Pirelli et al., New Eng. J. Med., 2000; Iacoviello et al., N. Eng. J. Med. 1998~~).
L <20,21>

15

DESCRIPTION OF THE INVENTION

The present invention relates to a molecule of nucleic acid comprising a sequence of the gene that codes for factor VII, characterized in that said molecule includes at least one allelic variant, said allelic variant affecting to the stability and/or functionality of said nucleic acid molecule, of the product obtained by transcription of said nucleic acid molecule and/or of the product coded by said nucleic acid molecule.

In the present invention, "nucleic acid molecule" is taken to mean a DNA sequence from the gene coding for factor VII protein. The length of said sequence is not an essential or restrictive aspect of this invention.

In the present invention, "allelic variant" is taken to mean a genetic variation in the DNA sequence that codes for factor VII protein, said genetic variation involving a pathology, loss or gain of stability and/or

[20]= Girelli D, Russo C, Ferraresi P, Olivieri O, Pinotti M, Friso S, et al. Polymorphisms in the factor VII gene and the risk of myocardial infarction in patients with coronary artery disease. N.Engl.J.Med. 2000; 14: 774-80

[21]= Iacoviello L.; DiCastelnuovo A.; DeKnijfff P.; D' Orazio A., Amore C., Arboretti R. et al Polymorphisms in the coagulation factor VII gene and the risk of Myocardial infarction. N. Eng. J. Med. 1998; 338: 79-85.

CLAIMS

1. Molecule of nucleic acid which comprises a sequence of the gene that codes for factor VII, characterized in that said molecule includes at least one allelic variant, said allelic variant ~~affecting the stability and/or functionality of said nucleic acid molecule and/or of the product coded by said nucleic acid molecule~~

10

~~2. Molecule of nucleic acid according to Claim 1, characterised in that the presence of at least one of said allelic variants is indicative of a predisposition to a cardiovascular disease.~~

15

~~3. Molecule of nucleic acid according to Claim 1 or 2, in which said allelic variant is one of those identified in Table 1:~~

<insert pages 16a and 16b>

20 <1> 4. Isolated product coded by a nucleic acid molecule according to ~~any of~~ Claim 1 ~~to 3~~, for use as a medicament.

<3> 5. Allele-specific oligonucleotide which hybridizes with a nucleic acid molecule as claimed in ~~any of~~ Claim 1 ~~to 3~~, in which the nucleotide of the polymorphic locus of said allele-specific oligonucleotide is different from the nucleotide of the polymorphic locus of the reference allele.

30

<3> <4> 6. Oligonucleotide as claimed in Claim 1 5, characterised in that it is a probe.

Table 1: allelic variants identified in the present invention. SNP (Single Nucleotide Polymorphism) variation of a base (nucleotide) in the DNA sequence.

Nucleotide O'Hara et al.	Allelic Variant	Position	Type
-3216	C/T	Promoter	SNP
-2987	C/A	Promoter	SNP
-668	A/C	Promoter	SNP
-628	A/G	Promoter	SNP
-402	G/A	Promoter	SNP
401	G/T	Promoter	SNP
323	Ins 0/10	Promoter	Insertion
122	T/C	Promoter	SNP
73	G/A	Intron 1	SNP
260	A/G	Intron 1	SNP
364	G/A	Intron 1	SNP
698	T/C	Intron 1	SNP
705	G/A	Intron 1	SNP
710	C/G	Intron 1	SNP
723	IVS1	Intron 1	VNTR
799	T/C	Intron 1	SNP
806	G/A	Intron 1	SNP
811	C/G	Intron 1	SNP
833	T/C	Intron 1	SNP
3.171	G/A	Intron 2	SNP
3.294	G/A	Intron 2	SNP
3.380	C/T	Intron 2	SNP
3.423	G/T	Intron 2	SNP
3.928 Q35Q	G/A	Exon 3	SNP
4.003	G/A	Intron 3	SNP
5.191	A/G	Intron 3	SNP
5.503	T/A	Intron 3	SNP
6.331	G/A	Intron 5	SNP
6.448	G/T	Intron 5	SNP
6.452	G/T	Intron 5	SNP
6.461	IVS5	Intron 5	VNTR
7.161	G/C	Intron 5	SNP

7.453	T/G	Intron 5	SNP
7.729	G/A	Intron 5	SNP
7.880 H115H	C/T	Exon 6	SNP
8.695	G/A	Intron 6	SNP
9.724	IVS7	Intron 8	VNTR
9.734	A/G	Intron 8	SNP
9.779	T/C	Intron 8	SNP
9.792	G/A	Intron 8	SNP
9.847	C/T	Intron 8	SNP
10.524	G/A	Intron 8	SNP
10.534	T/C	Intron 8	SNP
10.799 A294V	C/T	Exon 9	SNP
10.914 S333S	G/A	Exon 9	SNP
10976 R353Q	G/A	Exon 9	SNP
11.293	Ins AA	3'-UTR	Insertion
11.622	Del AG	3'-UTR	SNP
11.912	G/A	3'-UTR	SNP

<5> 7. Oligonucleotide as claimed in Claim ^{<3>}1~~8~~, characterised in that it is one of ~~those identified in Table 3~~ ^{<3>}group consisting in SEQ ID N° 1 to 36).

5 <6> 8. Procedure for analysis of a nucleic acid molecule, characterised in that it comprises obtaining said molecule from biological sample and determining at least one allelic variant from Table 1, said allelic variant affecting the stability and/or functionality of
10 the nucleic acid molecule and/or of the product coded thereby.

<7> 9. Diagnostic device for determining a predisposition to a cardiovascular disease, characterised
15 in that it includes an oligonucleotide according to any of Claims ^{<3>}1^{<5>} to 7.

8. Use of a molecule of a nucleic acid according to claim 1 for the development of therapeutic, preventive or diagnostic approaches for the treatment of a cardiovascular disease.

9. Use of an isolated product according to claim 1 for the manufacture of a medicament for the treatment of a cardiovascular disease.

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

☒ **BLACK BORDERS**

☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**

☒ **FADED TEXT OR DRAWING**

☒ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**

☐ **SKEWED/SLANTED IMAGES**

☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**

☐ **GRAY SCALE DOCUMENTS**

☒ **LINES OR MARKS ON ORIGINAL DOCUMENT**

☒ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**

☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.